that SilB C-terminal domain could function as a metallochaperone to the SilABC system.

1291-Pos

Crystal Structure and Metal-Binding Specificity of ZneB, the Periplasmic Adaptor Protein of A Heavy Metal Resistance System from Cupriavidus Metallidurans CH34

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In Gram-negative bacteria, tripartite efflux systems are involved in the transport of a broad range of toxic compounds such as drugs or heavy metals out of the cell. These protein complexes span the entire bacterial cell envelope, and are composed of an inner membrane transporter belonging to the resistance nodulation cell division (RND) family, an outer membrane protein member of the Outer Membrane Factor (OMF) family, and a periplasmic adaptor protein, member of the Membrane Fusion Protein (MFP) family. The periplasmic adaptor plays an important role in the recruitment of the two integral membrane partners and for the assembly of a functional transport complex. It has been proposed that this component could also contribute to the binding of the substrate. We report here on the characterization of ZneB, the MFP of a Heavy Metal Efflux-RND system from Cupriavidus metallidurans CH34. Using mass spectrometry, we have demonstrated that ZneB has a high specificity for zinc binding with a metal stoichiometry of 1:1 to the protein. The protein was crystallized in the presence of zinc and the apo- and metallated-forms were detected in the same asymmetric unit. The involvement of two histidine and a glutamate residues in the metal ion coordination site was confirmed by site-directed mutagenesis. The comparison of apo- and Zn-bound conformations based on the crystal structures and on data obtained in solution reveals important conformational changes upon zinc binding, suggesting an active role of the MFP in the efflux mechanism. The characterization at the molecular level of the efflux system proteins and the comparison with their counterparts in homologous RND-type transport systems involved in multidrug resistance will allow a better understanding of the resistance mechanisms.

1292-Pos

Crystal Structure of the N-terminal Region of Brain Spectrin Reveals A Helical Junction Region and A Stable Structural Domain

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The crystal structure of a recombinant protein consisting of the first 147 residues of brain α spectrin was solved to 2.3 Å. The N-terminal region consists of the partial domain (Helix C') and the anti-parallel, triple helical coiled-coil first structural domain (helices A1, B1, and C1). The data revealed that each asymmetric unit contained two crystallographically independent structures (1 and 2). The crystal structure of the first structural domain resembled that of the first structural domain of erythroid α-spectrin, determined before by solution NMR studies, with some specific differences, especially at the N-terminal region, including Helix C' and the region connecting Helix C' with the first structural domain (the junction region). The first ten residues are in a disordered conformation, followed by Helix C' with an apparent, flexible bend. The junction region exhibits a helical conformation in contrast with an unstructured junction region in erythroid α spectrin. A special feature that has not been reported in other spectrin domains is the long and flexible A1B1 loop of 13 residues. This loop is likely the recognition site for interaction with other proteins. Hydrogen bonds and hydrogen bond networks were identified in the first structural domain and compared with those in erythroid α-spectrin. We suggest that these hydrogen bonds might contribute toward the stability of brain and erythroid spectrin.

1293-Pos

An Overlapping Kinase and Phosphatase Docking Site Regulates Activity of the Retinoblastoma Protein

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Insights into the molecular mechanisms that regulate the phosphorylation state and corresponding activity of the retinoblastoma tumor suppressor protein (Rb) are fundamental to understanding the control of cell proliferation. While much focus has been placed upon regulation of Cyclin-dependent kinase (Cdk) activity towards Rb, less is known about Rb dephosphorylation catalyzed by the major Rb phosphatase, protein phosphatase-1 (PP1). Using x-ray crystallography, we have determined the crystal structure of a PP1:Rb peptide complex to 3.2Å that reveals an overlapping kinase and phosphatase docking site. Kinetic assays show that Cdk and PP1 docking to Rb are mutually exclusive and that this docking site is required for efficient dephosphorylation, as well as phosphorylation of Rb. Cell cycle arrest assays demonstrate that the ability of PP1 to compete with Cdks is sufficient to retain Rb activity and block cell cycle advancement. These results establish a novel mechanism for the regulation of Rb phosphorylation state in which kinase and phosphatase compete for substrate docking.

1294-Pos

Crystal Structures Of Yeast Mitochondrial ATPase with Uncoupling Mutations

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Yeast mitochondrial ATP synthase is a transmembrane protein responsible for synthesis of more than 90% of ATP under aerobic conditions. The water soluble portion of ATP synthase, F1, is composed of five subunits with stoichiometry $\alpha_3\beta_3\gamma\delta\epsilon$ and has a combined molecular weight of 360 kDa. The three active sites of ATPase are formed at the interfaces between alternating α- and β-subunits. ATPase is capable of efficient ATP hydrolysis, accompanied by rotation of central stalk subunits, $\gamma\delta\epsilon$, within the $\alpha_3\beta_3$ core and is capable of ATP synthesis if central subunits are forced to rotate in the opposite direction. A number of mutations in ATP synthase have been identified that result in the uncoupling of catalytic function and proton flow across the mitochondrial membrane. These uncoupling mutations cluster at the interface between γ-subunit and $\alpha_3\beta_3$ catalytic core of ATPase. In this work, four X-ray crystal structures of ATPase with single amino acid substitutions αN67I, αF405S, βV279F, and γI270T were solved at resolutions ranging from 3.2 Å to 2.74 Å. This study will present a structural comparison of the mutant structures with the wild type structures to understand the mechanism of coupling. However, the crystal structures likely represent the ground state of catalytic reaction cycle while the mutations may result in notable distortions of enzyme structure during other stages of catalytic cycle. Additionally, the uncoupling of the ATPase may be caused by changes in the energy of interaction between the portions that are rotating in the molecular machine thereby altering transition to the higher energy states. Structural based hypotheses are presented to explain the role of these residues in the coupling of the enzyme. Supported by NIH R01GM0662223

1295-Pos

Molecular Mechanism of the Peptidoglycan Hydrolysis by FlgJ, A Putative Flagellar Rod Cap Protein From Salmonella

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The axial structure of the bacterial flagellum consists of three parts: the filament as a helical propeller; the hook as a universal joint; and the rod as a drive shaft connecting the hook and the rotor. The assembly of the axial structure, which occurs at its distal end, requires cap complexes attached to the growing end. FlgD and FliD are cap proteins necessary for hook and filament growth, respectively. For efficient penetration of the growing rod through the peptidoglycan (PG) layer, it is likely that the rod cap locally degrades PG. FlgJ is a putative rod cap protein with a PG-hydrolyzing activity (muramidase). Previous studies have shown that the N-terminal region of FlgJ interacts with the rod proteins and that the C-terminal region shows a sequence similarity to muramidase family, such as autolysin and AcmA. To understand the mechanisms of rod formation, we determined the atomic structure of a C-terminal fragment of FlgJ at 1.7 Å.

The crystal structure revealed the entire muramidase domain of FlgJ. In spite of no significant sequence similarity, the putative active site of FlgJ closely resembles that of hen egg white lysozyme (HEWL), which is a well-studied muramidase. A glutamic acid residue at position 184 in FlgJ is invariant among FlgJ family and the E184Q mutant of FlgJ shows no muramidase activity, indicating